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An Optimal Cell Detection Technique for Automated Patch Clamping

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Abstract

While there are several hardware techniques for the automated patch clamping of cells that describe the equipment apparatus used for patch clamping, very few explain the science behind the actual technique of locating the ideal cell for a patch clamping procedure. We present a machine vision approach to patch clamping cell selection by developing an intelligent algorithm technique that gives the user the ability to determine the "good" cell to patch clamp in an image within one second. This technique will aid the user in determining the best candidates for patch clamping and will ultimately save time, increase efficiency and reduce cost. The ultimate goal is to combine intelligent processing with instrumentation and controls in order to produce a complete turnkey automated patch clamping system capable of accurately and reliably patch clamping cells with a minimum amount of human intervention. We present a unique technique that identifies "good" patch clamping cell candidates based on feature metrics of a cell's (x, y) position, major axis length, minor axis length, area, elongation, roundness, smoothness, angle of orientation, thinness and whether or not the cell is only particularly in the field of view. A patent is pending for this research.

Introduction

Patch clamping is a widely applied electrophysiological technique for the study of ion channels, membrane proteins that regulate the flow of ions across cellular membranes and therefore influence the physiology of all cells. Patch clamping is used in many biological research fields such as neurobiology, pharmacology and molecular biology. Patch clamping was introduced by Neher and Saskmann (1991) in 1976 for recording the current in a small patch of membrane under voltage-clamp conditions and Neher developed the gigaseal, which is used widely today for patch clamping applications. The high resolution in time and current are the main advantages of the patch clamping technique. Gorelik, et al. (2002) discuss a Smart Patch clamp system that can be used to study ion channels is cellular structure in the patch clamping process. Stett, et al. (2003) explain the use of an automated patch clamping system that utilizes a non-conventional method of path clamping for basic cellular research. Sigworth and Klemic (2002) introduce a novel approach to developing and implementing a patch clamp on a chip method that can be used to revolutionize the field of patch clamping. Lepple-Weinhues, et al. (2003) discuss a high quality yet cost-efficient path clamp screen that can be used for in the patch clamping process. Most of the current published research into automated patch clamping discuss primarily the hardware aspect of patch clamping.

During a patch clamping procedure, an electrode is sealed to a cell membrane while a small patch of membrane is electrically isolated and currents through it are determined. The currents through one ion

channel can be recorded and the characteristics of one protein molecule can be determined. Patch clamping can be carried out on patches of a membrane that have been removed from the cell, either as "inside-out" (the pipette is gently pulled away from the cell) or "outside-out" (pipette is gently pulled away from the cell causing the membrane to rupture and the free ends to reseal) patches, or it can be performed on an intact cell. A pipette with a micron tip diameter is pressed against a living cell to form a seal, called a "gigaseal" because of its high resistance, and gentle suction is applied. Once the current flowing through the pipette is the same as that flowing through the membrane and a known voltage is applied across the membrane, high resolution measurements of the current can be obtained (Molleman, 2003).

An example of a patch clamping technique is described in detail by Molleman (2003).

There are several companies that produce patch clamping equipment ranging from low-cost solutions for student laboratories, amplifiers optimized for the lowest noise when measuring single channel events and highly efficient systems for whole cell patch clamping. While there are patch clamping hardware solutions available commercially, what is not available is a generally accepted method for determining what cells to choose as "good" candidates for patch clamping. Our technique aims to provide such a method in order to increase the frequency and accuracy of determining and obtaining a successful patch clamp automatically. By eliminating the human factor and incorporating a machine vision technique, an optimal patch clamping technique can be incorporated into any existing patch clamping experimental or laboratory setup.

We have developed a feature-based imaging technique that will automatically determine the "good" cells to attempt a patch clamp in a field of view by determining each cell's (x,y) position, area, major axis length, minor axis length, elongation, smoothness, roundness, thinness, partiality and angle of orientation. A human operator does not perform at peak efficiency throughout the day, but our technique can effectively be at peak efficiency non-stop. By assisting or augmenting a human operator in determining the "good" patch clamping candidates, one can save time, increase efficiently and more importantly, save money.

It is now possible to automatically control via automated instrumentation and control techniques each function of the patch clamping hardware process and it is possible to combine this with our optimal cell detection technique to arrive at a complete turnkey automated patch clamping technique that can be used in a laboratory setting. We initially relied on human operator input to determine criteria for cell classification. We then optimized this technique and developed an automated solution specifically for patch clamping. What follows is a description of our optimal cell detection technique as well as examples.

Materials and Methods

The goal of this paper is to present a novel technique on optimal cell identification for patch clamping utilizing commercial off-the-shelf patch clamping hardware. No specific hardware is needed for the successful implementation of our technique. A standard patch clamping microscope system with a CCD camera attached, a personal computer with an image processing board and software development experience are all that is needed. The cell image is first captured by the microscope CCD camera and image processing board. Then, the image data is analyzed by our machine vision technique and the result is the identification of "good" cell candidates for patch clamping. Once a "good" cell is identified, a successful patch clamp can be implemented by an automated patch clamping system or a human operator.

We developed this technique to be used for a actual patch clamping laboratory. From independent patch clamping observations of laboratory technicians, technology decision meetings and talks with patch clamping operators, the following criteria emerged as important in the human cognitive process of classifying cells as good or bad candidates for a patch clamping operation.

Good Cell Characteristics:

- Triangular or oval shape
- Single cells
- Average size or above
- Smooth contour
- Separate
- Multiple alive and growing processes (spreading tentacles are actively searching the surface to find other cells)
- Indistinct nucleus Smooth gradient for nucleus

Bad Cell Characteristics:

- Round or skinny shape
- Dividing cells or clumps
- Very small in relation to surrounding cells
- Wrinkled, uneven or ragged edges
- Touching or very near other cells
- No processes or stumpy processes
- Distinct nucleus
- Distinct lines in nucleus means multiple cells

We determined the minimum set of cell feature metrics that would reliably classify cells into the categories of "good" and "bad" and developed an automated technique to generate and read the metric data files and provide cell classification. The simplest set of metrics turned out to be partial, major axis length, roundness, and thin in center. Other metrics such as smoothness, elongation, and nearest neighbors were not needed for the initial classification but could serve to refine the routine and determine best candidates. Metrics tests could be developed for some of the other criteria such as nucleus appearance if further refinement proved useful. We used the following decision tree for our technique:

Step 1: Automated algorithm reads in data file (id, x, y, majorAxis, minorAxis, area, elongation, roundness, smoothness, theta, thinInCenter, partial)

Step 2: Bad or Good classification

Classified as bad if:

- Particles are partials (cannot determine correct metrics if only part of the particle is in the field of view)
- Particles whose major axis is too small (major axis < 50.0)
- Particles that are too round (roundness > 0.5)
- Particles that get thin in the center (indicates a dividing cell)

Otherwise:

- Classified as good

Based on the decision tree listed in Step 1 and Step 2, we can determine "good" or "bad" cells used for patch clamping. The goal is to classify all "good" cells in an image to increase the probability of a successful patch clamp, which may require an attempt at several cells in an image.

Image Preprocessing

Before we can make a decision based on the cell image, we first need to conduct pre-processing on the cell image in order to eliminate any potential errors and distortions. The overall goal is to identify all objects in the cell image based on the following metric feature set:

Metrics

- Roundness
- Elongation
- Thin in center (indicates a dividing cell)
- Major axis length
- Perimeter smoothness (low number indicates smoother particle)
- Nearest neighbor

Figure 1 is an example of a potential patch clamping candidate. In order to identify and classify cells, we must preprocess the images to isolate the cells from the background. For the patch clamping images, we used two parallel preprocessing paths. The first path uses a histogram stretch to improve the image contrast paired with a Sobel filter that highlights edges by finding changes in intensity (fig. 2). A threshold operator was then used to eliminate small intensity changes. This approach clearly identified particle boundaries but did not always preserve cell connectivity since cell perimeters often had gaps.

The second approach used a custom designed variance filter. As the filter passes over the image, the variance is computed as $s^2 = \frac{1}{N}\sum_{i=1}^{N}(x_i - \bar{x})^2$, where N is the number of pixels and $\bar{x}$ is the average in each neighborhood. If the neighborhood of the center pixel has a low variance, the area is basically constant and the center pixel is set to black. If the neighborhood has a high variance, intensity values are changing over the neighborhood and the center pixel is left unchanged. This approach preserves the connectivity of the cells and has no corruption of cell data, but it also includes some of the background as part of the cell (fig. 3). We determined that the optimal technique was to combine the two approaches giving an image where the cells are visually enhanced without losing connectivity (fig. 4).

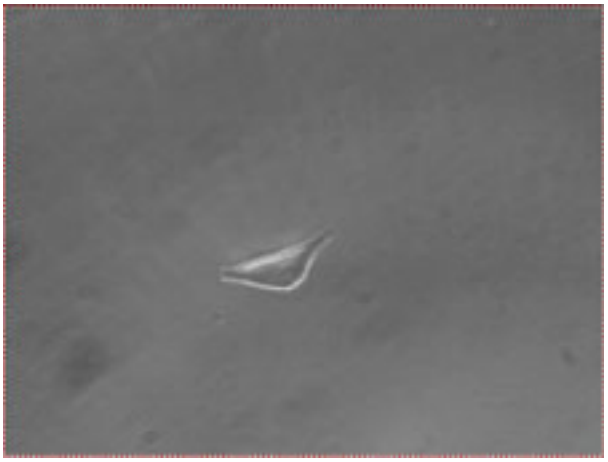

Figure 1.—Ideal cell image for patch clamping.

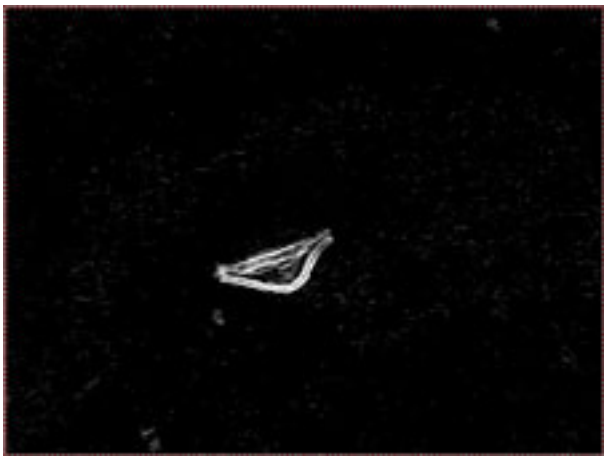

Figure 2.—Histogram and Sobel filter applied to cell image

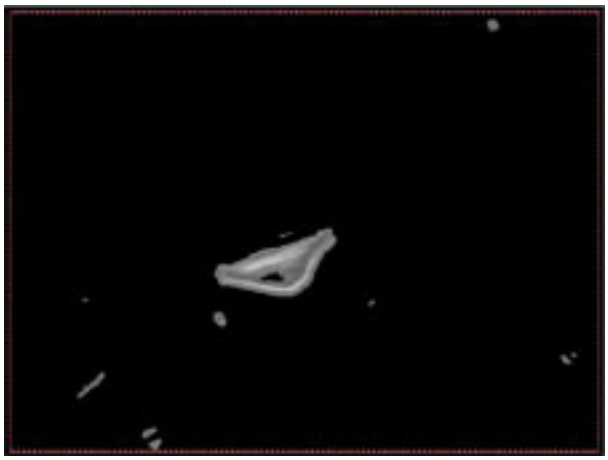

Figure 3.—Variance filter applied to cell image.

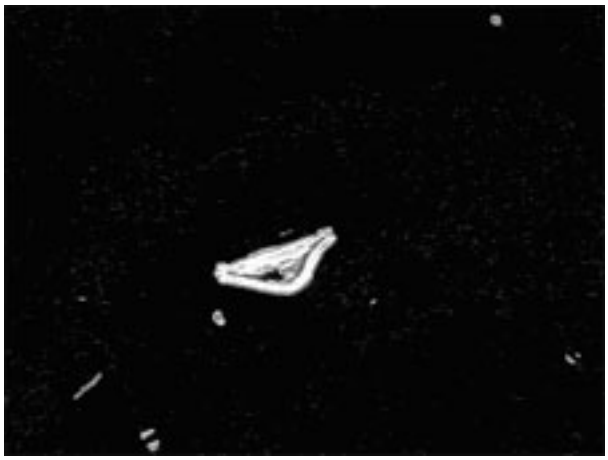

Figure 4.—Histogram and Sobel filter combined with variance filter applied to cell image.

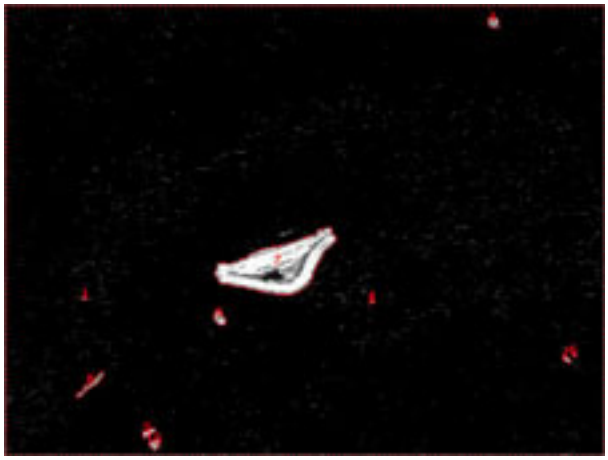

Figure 5.—Result of find particles technique.

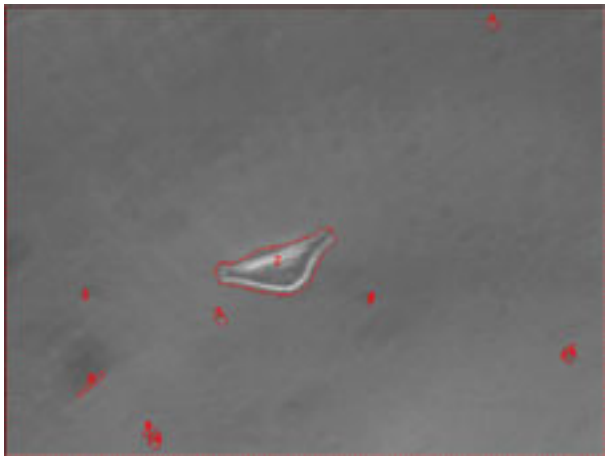

Figure 6.—Overlay of identified objects from original cell image.

We can then run the find particles technique to identify all the non-background objects present in the image (fig. 5). An overlay of the object perimeters and labels is shown on the original image (fig. 6).

Once we identify all the objects in an image, they are analyzed to produce the metrics we will use for classification as shown in the table 1:

TABLE 1.—IMAGE DATA FROM FIGURE 5.

id	x	y	major	minor	area	elong	round	smooth	theta	thin	prtl
1	517	18	14.9	8.8	117	0.589	2.169	0.187	98	F	F
2	**289**	**275**	**124.5**	**66.5**	**4882**	**0.534**	**0.178**	**0.143**	**22.6**	**F**	**F**
3	84	310	3.5	2.7	16	0.776	0.88	0.154	31.7	F	F
4	388	313	6.4	3.2	24	0.495	0.93	0.183	37	F	F
5	227	330	16.7	11.2	155	0.67	0.967	0.16	114.9	F	F
6	603	369	4.7	3.2	21	0.674	1.817	0.115	135	F	F
7	595	374	11.9	6.1	62	0.515	0.697	0.19	137.6	F	F
8	91	401	37.8	9.4	202	0.249	0.232	0.161	42.2	F	F
9	152	451	16.4	7.6	99	0.462	0.535	0.169	26.1	F	F
10	159	463	13.7	9.9	106	0.723	0.898	0.174	43.5	F	F

Note: 2 is the “best” candidate for patch clamping

Based on the information listed in table 1, we can classify an ideal patch clamping candidate by its major axis, minor axis, area, elongation, roundness, smoothness, theta (orientation with respect to a coordinate system), thinness, and whether the object is completely contained in the field of view or only partially contained (partial).

Results and Discussion

Based on identifying the feature metrics of all objects in a cell image, we can determine a “good” or “bad” cell for a typical patch clamping procedure. Using a complex image such as the image listed in figure 7 and applying our histogram and Sobel filter combined with variance filter technique, we get the following results shown in figures 8 to 10. The patch clamping data from figure 7 is listed in table 2. By looking at the results listed in table 2, cell 15 is the only “good” candidate based on the metrics used to determine an optimal cell for patch clamping.

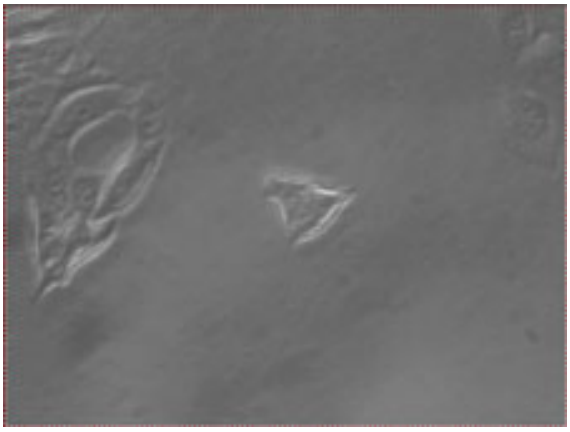

Figure 7.—Complex cell image for patch clamping.

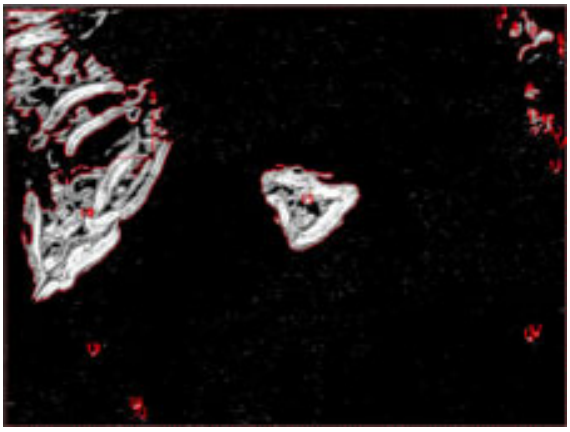

Figure 8.—Result of find particles technique.

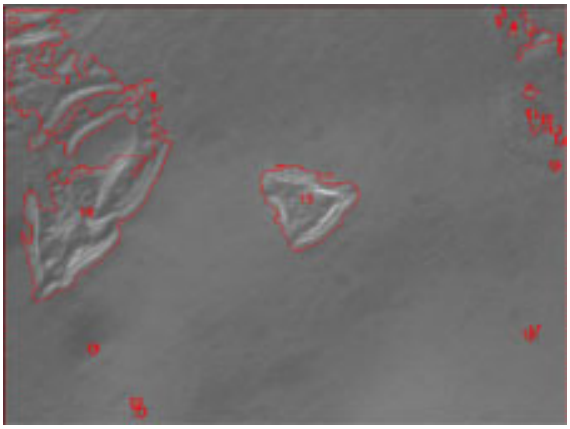

Figure 9.—Overlay of identified objects from complex cell image.

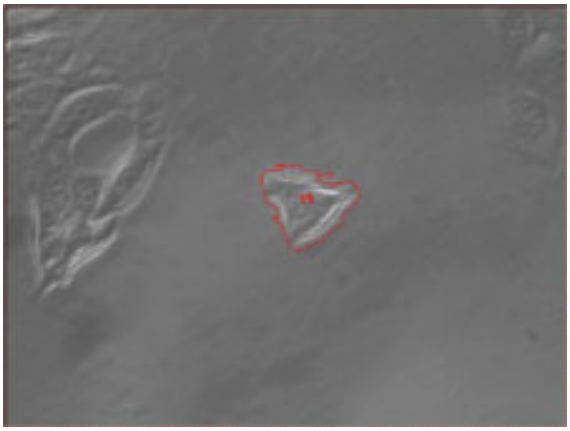

Figure 10.—"Good" cell candidate for patch clamping.

TABLE 2.—IMAGE DATA FROM FIGURE 7.

id	x	y	major	minor	area	elong	round	smooth	theta	thin	prtl
1	72	89	191.8	126.7	12491	0.661	0.077	0.143	137	F	T
2	597	33	49.9	44.9	844	0.899	0.18	0.162	1.2	T	T
3	565	12	6.8	3.7	27	0.537	0.908	0.203	40.3	F	F
4	575	28	17.3	9.3	114	0.536	0.449	0.1	83.3	F	F
5	559	21	4	2.5	15	0.615	0.943	0.159	80.8	F	F
6	629	41	3.4	2.3	13	0.676	0.99	0.205	103.3	F	T
7	629	51	12	4	38	0.333	0.489	0.144	90	T	T
8	595	98	16	14	175	0.876	0.884	0.168	4.7	F	F
9	169	108	4	4	19	1	1.775	0.167	0	F	F
10	93	239	247.1	117.3	14243	0.475	0.093	0.139	44.7	T	F
11	599	129	35.8	14.6	277	0.409	0.323	0.185	99.4	F	F
12	614	132	22.1	5.5	121	0.25	0.361	0.161	76.5	F	F
13	623	146	5.4	1.9	15	0.346	0.619	0.135	74	F	F
14	630	158	3.2	1	7	0.302	0.752	0.188	74.5	F	T
15	**342**	**223**	**122**	**88.4**	**6417**	**0.73**	**0.3**	**0.14**	**161**	**F**	**F**
16	624	185	6.7	4.1	26	0.618	0.965	0.2	58.3	F	F
17	601	371	5.1	3.8	21	0.744	1.805	0.188	125.8	F	F
18	594	377	6.7	5	31	0.739	1.269	0.203	137.4	F	F
19	101	392	8	4.6	34	0.572	0.994	0.147	36	F	F
20	150	453	9.4	5	50	0.535	0.845	0.125	26.6	F	F
21	155	465	5.4	2.2	15	0.396	0.75	0.25	5.7	F	F

Note: 15 is the "best" candidate for patch clamping

Using a second complex image such as the image listed in figure 11 and applying our histogram and Sobel filter combined with variance filter technique, we get the following results shown in figures 12 to 14. The patch clamping data from figure 11 is listed in table 3. By looking at the results listed in table 3, there are no "good" candidates based on the metrics used to determine an optimal cell for patch clamping. This is a significant advancement in the field of patch clamping because we now have to ability to automatically scan through a sample dish and identify ideal cell candidates with no human interaction. The technique has proven to be more efficient over time than an actual human who has to look at each and every cell sample over a period of hours.

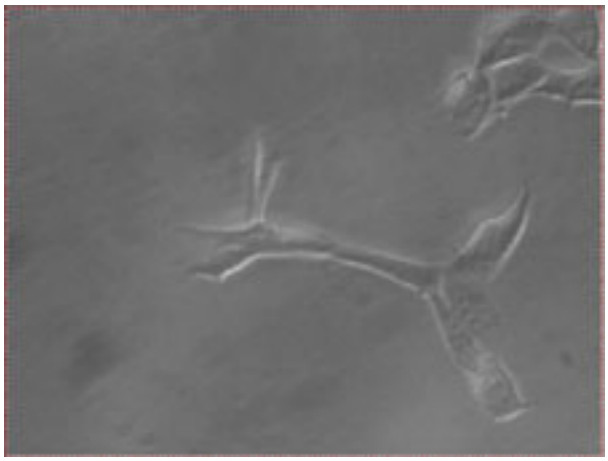

Figure 11.—Second complex cell image for patch clamping

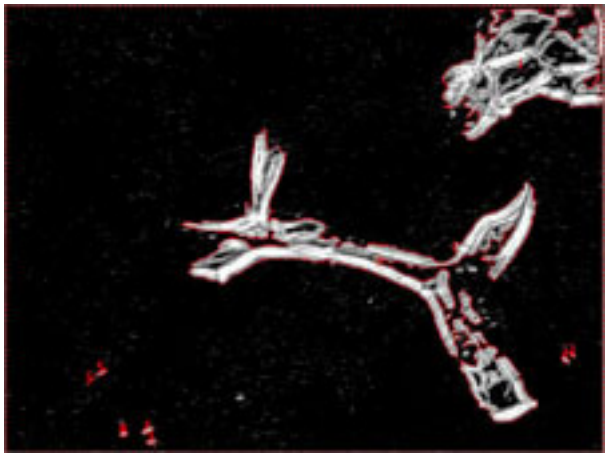

Figure 12.—Result of find particles technique

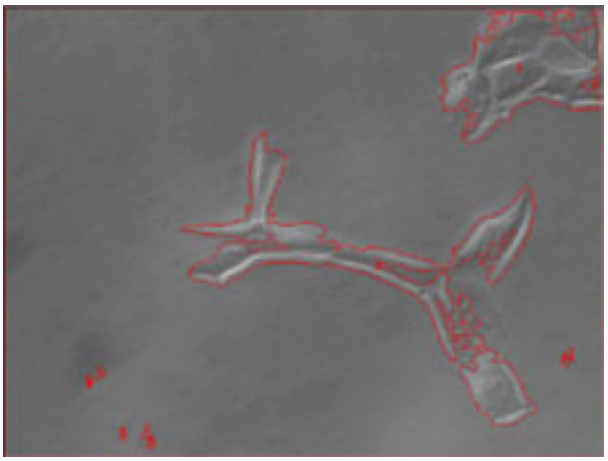

Figure 13.—Overlay of identified objects from second complex cell image.

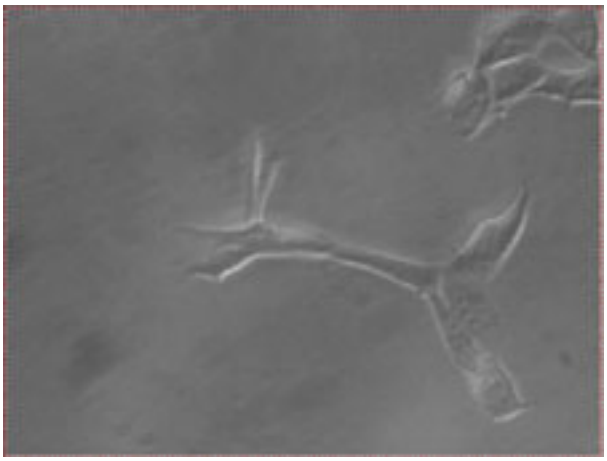

Figure 14.—"Good" cell candidate for patch clamping.

TABLE 3.—IMAGE DATA FROM FIGURE 12.

id	x	y	major	minor	area	elong	round	smooth	theta	thin	prtl
1	547	69	214.1	104	12017	0.486	0.154	0.133	9.9	F	T
2	398	279	448.5	202.4	21363	0.451	0.048	0.14	145.5	T	F
3	6.2	372	6.9	3.9	30	0.566	1.134	0.125	118.2	F	F
4	594	377	11.1	6.6	58	0.588	0.991	0.174	150.5	F	F
5	102	391	12.8	5.8	63	0.458	0.557	0.188	27.2	F	F
6	91	400	8.5	3.4	21	0.393	0.44	0.264	44	F	F
7	151	452	13.5	6.4	77	0.474	0.634	0.164	27.8	F	F
8	126	456	7.9	4.7	43	0.599	1.03	0.113	22.5	F	F
9	155	464	10.7	5.2	55	0.484	0.764	0.18	14.9	F	F

Note: There is no “good” candidates for patch clamping

Conclusion

A machine vision approach for automatically determining an optimal cell for patch clamping has been presented and discussed. This technique utilizes known cell feature metrics such as cell size, shape, contour, nucleus and tentacle structure to accurately make decisions on cell patch clamping optimality within one second. Combined with automated patch clamping hardware, this technique can be used to develop a turn-key automated patch clamping system that will ultimately save time, increase efficiency and reduce cost. This optimal cell detection technique has been shown to be capable of capable of accurately and reliably identifying good and bad cell candidate for patch. Using the results can lead to the development of a highly accurate and reproducible automated patch clamping system.

References

Fertig N., Blick, R.H., and Behrends J.C., 2002. Whole Cell Patch Clamp Recording Performed on a Planar Glass Chip, Biophys J, pp. 3056–3062, vol. 82, no. 6.

Gorelik J., Gu Y., Spohr H.A., Shevchuk A.I., Lab M.J., Harding S.E., Edwards C.R.W., Whitaker M., Moss G.W.J., Benton D.C.H., Sánchez D., Darszon A., Vodyanoy I., Klenerman D., and Korchev Y.E., 2002. Ion Channels in Small Cells and Subcellular Structures Can Be Studied with a Smart Patch-Clamp System Biophys. J., pp. 3296–3303, vol. 83, no. 6.

Lepple-Wienhues A., Klaus Ferlinz K., Seeger A., Schäfer A., 2003, Flip the Tip: An Automated, High Quality, Cost-Effective Patch Clamp Screen, Receptors and Channels, 9:13–17.

Molleman A., 2003. Patch Clamping: An Introductory Guide to Patch Clamp Electrophysiology, John Wiley & Sons Ltd. ISBN: 047148685X.

Neher E. and Sakmann B., 1991. The Nobel Prize in Physiology or Medicine 1991 for their discoveries concerning the function of single ion channels in cells.

Sigworth F.J. and Klemic K.G., 2002. Patch Clamp on a Chip, Biophys J, pp. 2831–2832, vol. 82, no. 6.

Stett A., Burkhardt C., Weber U., van Stiphout P., and Knott T (2003). Cytocentering: A novel technique enabling automated cell-by-cell patch clamping with the CytopatchTM chip. Receptors and Channels, 9(1):59–66

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13. ABSTRACT ***(Maximum 200 words)***

While there are several hardware techniques for the automated patch clamping of cells that describe the equipment apparatus used for patch clamping, very few explain the science behind the actual technique of locating the ideal cell for a patch clamping procedure. We present a machine vision approach to patch clamping cell selection by developing an intelligent algorithm technique that gives the user the ability to determine the "good" cell to patch clamp in an image within one second. This technique will aid the user in determining the best candidates for patch clamping and will ultimately save time, increase efficiency and reduce cost. The ultimate goal is to combine intelligent processing with instrumentation and controls in order to produce a complete turnkey automated patch clamping system capable of accurately and reliably patch clamping cells with a minimum amount of human intervention. We present a unique technique that identifies "good" patch clamping cell candidates based on feature metrics of a cell's (x, y) position, major axis length, minor axis length, area, elongation, roundness, smoothness, angle of orientation, thinness and whether or not the cell is only particularly in the field of view. A patent is pending for this research.

14. SUBJECT TERMS

Automated patch clamping; Cell detetion; Cell classification; Biomedical imaging; Machine vision; Imaging processing

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